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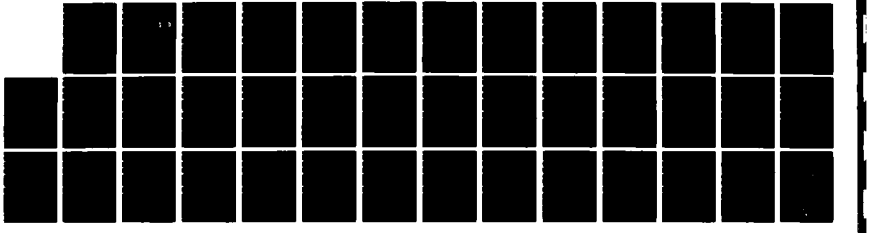

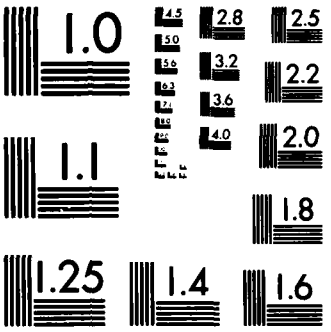

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FACTORS INFLUENCING CARBOXYHEMOGLOBIN STABILITY

FINAL REPORT

George M. Goldstein[1]
Louis Raggio[2]
Dennis House[1]

26 March 1985

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, MD 21701-5012

Project Order 1811

[1]United States Environmental Protection Agency
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800 Eastowne Drive, Suite 200
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Fort Detrick, Ferderick, MD 21701

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Studies were conducted to determine the stability of carboxyhemoglobin (COHb) in evacuated blood containers using the IL-282 co-oximeter as the measurement instrument. This study has shown that COHb levels decrease by 5 to 10% of the original value after three days of storage and then remained stable for 14 days at 4°C or 21°C in vacutainers containing heparin or EDTA. The storage temperature, 4°C or 21°C had no appreciable affect on COHb levels. Blood sam-

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ples that contained the anticoagulant heparin had higher initial values of COHb than samples with EDTA. In this study, ambient room light levels did not affect the measured levels of COHb at 4°C for 5 days.

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CO	Carbon monoxide
COHb	Carboxyhemoglobin, a complex of carbonmonoxide and hemoglobin found in the blood
EDTA	Ethylenediamine tetraacetic acid, a chelating agent used as a blood anticoagulant in stored blood samples
P	Probability
H	Hypothesis
T	Time
TE	Temperature
AC	Anticoagulant
MetHb	Methemoglobin, hemoglobin molecule where the iron moiety has been oxidized
$K_3Fe(CN)_6$	Potassium ferric cyanide

INTRODUCTION

Carbon monoxide (CO) is a byproduct of the incomplete combustion of hydrocarbons. Because of its high affinity for oxygen binding sites on hemoglobin and displacing oxygen, the presence of CO in the atmosphere has been shown to produce adverse health effects, affecting the cardiovascular system as well as causing behavioral changes. These changes have been reported at concentrations of carboxyhemoglobin (COHb) in the range of 4 to 6% (1).

The Department of Defense (DOD) is concerned about the production of CO in fixed and mobile weapon systems and the relationship between behavior and health effects in military personnel associated with this exposure. The source of CO in a combat situation is exhaust gases from motorized vehicles and propellant gases from weapon systems. Bearing in mind the potential health and behavioral effects associated with the exposure of military personnel to CO, the DOD has established design specifications for weapon systems which limit the levels of CO to which military personnel may be exposed (2,3).

The effectiveness of these design criteria may be assessed by measuring the levels of CO that are produced during a military battlefield simulation and to quantitate the uptake of CO to which the individual has been exposed. Because continuous monitoring for CO is impractical, blood levels of carboxyhemoglobin (COHb) have been used to provide an integrated dose of CO exposure. When CO is inspired, it is absorbed through the lungs and combines with circulating hemoglobin in the blood to form COHb. COHb has been shown to be an accurate measurement of the mean CO exposure (4).

To test the design specifications in several weapons systems, as they pertain to the buildup of CO within those systems, the Department of the

Army recently proposed a study in which the CO concentrations generated in these weapon systems during actual field use would be estimated. These estimates of CO exposure were to be calculated from alveolar breath samples and blood samples taken from volunteers using these weapon systems. The blood samples were to be sent under refrigeration from Fort Benning, Georgia to the Environmental Protection Agency laboratory in Chapel Hill, North Carolina. The collection and shipment of blood samples was to occur over a four day period (7).

Blood samples have been shown to be stable for several weeks when used to quantitate COHb, depending upon sample storage conditions and type of analytical procedure used to quantitate the COHb (4-6). The experience gained with our recent acquisition of the Instrumentation Laboratory IL-282 CO-Oximeter (Instrumentation Laboratory, Lexington, MA 02173), an instrument used to quantitate COHb, demonstrated that blood samples may not be as stable as recently reported (4-6). Although Dennis and Valeri (11) reported on an extensive evaluation of the accuracy and precision capabilities of the IL-282 CO-Oximeter, there have been no reports of a comprehensive evaluation of factors associated with and affecting the blood sample integrity, which may subsequently affect measured values of COHb.

The factors that affect the stability of blood samples and its relationship to the estimation of COHb when measuring COHb by the spectrophotometric method employed by the IL-282 CO-Oximeter, led us to investigate factors that could affect the measurement of COHb. These factors include sample temperature (4-6), the choice of anticoagulant (8), the amount of storage time (4-6), and the effect of photon induced decomposition of COHb (9).

This report deals with the estimation of the levels of COHb in blood

under varing conditions (temperature, time, anticoagulant, and ambient light).

METHODS:

Blood Samples: Blood samples were obtained from male subjects ranging in age from 18 to 35 years. Each subject reported to smoke at least 1.5 packs of cigarettes per day. This level of smoking was estimated to produced moderate blood levels of COHb ranging from approximately 4 to 6% COHb. Blood samples were obtained in 3 ml pediatric vacutainers.

Instrument Calibration: In this study, all blood COHb determinations were made using an IL-282 CO-Oximeter. Calibration of the IL-282 was performed weekly, following the manufacturer's specifications. The hemoglobin values used to set the hemoglobin channel of the CO-Oximeter were determined by the cyanomethemoglobin method using a Coulter Hemoglobinometer model HGBR (Coulter Electronics, Inc., Hialeah, FL). The hemoglobinometer was calibrated weekly according to manufacturer's specifications using Fisher Diagnostics 501 Series Standards. Blood samples were brought to room temperature prior to analyses. All COHb values reported are the mean value of triplicate analyses.

Experimental Design: This study was designed to determine if a blood sample exposed to fluorescent room light, stored under varying storage temperatures and different anticoagulants, caused a change in the level of COHb over a period of time. The option of three sequential experiments was chosen over one experiment to address the four variables in question. This approach was chosen because the one experiment approach would generate a large number of blood samples that would require several hours for analysis. With time as one of the variables, it would be difficult to interpret the

results for that variable. Additionally, if blood for each variable was taken from the same subject in the subject pool, this would require each subject to donate approximately 170 milliliters of blood divided into fifty six pediatric vacutainers. It was felt that this procedure may require multiple venipunctures, making it unacceptable to the subject.

COHb determinations made by the CO-Oximeter are based upon the absorbance characteristics of the blood sample. Factors that affect these characteristics will have an effect on the estimation of COHb. The stability of carboxyhemoglobin in blood samples stored in an evacuated tube was investigated under conditions of varying the anticoagulant choice between heparin and ethylenediamine tetraacetic acid (EDTA); the storage temperature between 4°C and 21°C; and the length of storage time between 0 and 14 days.

In the first experiment of this study, two hundred eighty blood samples containing COHb were obtained from forty volunteers over a period of several weeks. These samples were divided into each of four combinations of temperature (4°C and 21°C) and anticoagulant (sodium heparin and potassium EDTA). For each subject anticoagulant/temperature combination, seven pediatric vacutainers (3 ml) of blood were obtained for analyses. One tube from each set was analyzed immediately. The remaining samples were analyzed during the next two weeks out to a 14 day determination. The times chosen for analyses were chosen to satistify the work schedule. The experiment was a three-factor (temperature, anticoagulant and time) experiment with repeated measurements on the time factor (Table 1).

The second experiment of the study was designed to determine if there was any effect of photo decomposition of COHb levels over time. A secondary question that was addressed dealt with the effect of anticoagulant choice. In this experiment, blood from each of nine subjects was placed into 12

pediatric vacutainers, six containing either the anticoagulant heparin or EDTA. Half of the anticoagulant group (3 tubes) were wrapped in aluminum foil to exclude light while the other three tubes were left exposed to ambient fluorescent room light. Of the three tubes in each anticoagulant wrapped and unwrapped combination, one was assayed immediately (day 0), one on day 3, and the last one on day 5. This experiment was a three-factor (anticoagulant, wrapped/unwrapped and time) study with repeated measures on all factors (Table 2). All samples were stored at 4°C. All samples were exposed to ambient light conditions for several hours prior to analyses.

The third experiment of the study was of similar design to the second experiment, in that blood from the same subject was divided across each of the measured variables. The primary focus of this experiment was the effect of temperature (4°C and 21°C) on sample stability, and secondarily, time (0, 3, and 5 days), and the choice of anticoagulant (heparin, EDTA). Blood from each of eleven subjects was placed into 4 pediatric vacutainers. These tubes were then assayed for COHb on day 0, 3 and 5 to determine the influence of the four possible combinations of temperature and anticoagulant. The design of this study was a three-factor experiment with repeated measures on all factors (Table 3).

For each experiment, multivariate analysis of variance methods were used to test for significance of effects. If an effect was significant and had more than two levels, Scheffe's multiple comparison test was done to determine which levels were different from each other.

RESULTS

Experiment 1

For the first experiment, the initial analysis of the data by multivariate analysis of variance revealed that there was no change in COHb

levels based upon the presence of either heparin or EDTA nor in samples stored at 4°C or 21°C. There was a change in COHb; however, that was time dependent ($P<0.001$) (Table 4). Table 5 depicts the mean COHb values across the four combinations of anticoagulant and temperature over 14 days of storage. Table 6 shows the mean values of pooled samples for day 0 through day 14. Scheffe's multiple-comparison procedure was used to determine which of the seven means of time over the 14 day study were significantly different from each other. The conclusion was that the mean from day 0 was significantly larger than the means at all other days and that the means at day 3 through day 14 were not significantly different from each other ($p<0.05$).

In addition to the main question of COHb stability, estimates of the variability of the COHb measurements were made over the range of 0 to 17% COHb. The mean value of triplicate measurements for each subject at each time interval was computed and then the variability of the corresponding three measurements was assigned to the category that included the mean of the three measurements. The hypothesis, H1, that the variance in the six categories (<2, 2 to <3, 3 to <4, 4 to <5, 5 to <9, and >9% COHb) were equal was tested using Bartlett's test. The hypothesis was rejected ($p=0.014$). In addition, the hypotheses that the variances in the first four categories were equal, H2, and that the variances in the last two categories were equal, H3, were tested. Neither of these hypotheses could be rejected. These results together imply that the variability of COHb measurements is approximately constant for COHb levels up to 5% (SD=0.148). For COHb levels of 5 to 17%, the variability is also constant, but larger than for COHb levels up to 5% (SD=0.183)(Table 7).

Experiment 2:

For the second experiment, multivariate analysis (Table 8) indicated that time and anticoagulant main effects are significant, ($P<.042$ for time and $P<.001$ for anticoagulant). The wrapped/unwrapped tubes were not significantly different from each other ($P<.914$) indicating that ambient fluorescent light did not cause dissociation of the COHb molecule. The mean COHb was significantly higher, however, in tubes containing heparin as the anticoagulant than in tubes containing EDTA (Table 9). Scheffe's multiple comparison test was done on the three time means (0, 3 and 5 days) to determine which time period was significantly different from the others. The conclusion drawn from this test is that the COHb mean at time 0 is significantly higher than the time 3 and time 5 sample means and that the time 5 sample mean is significantly higher than the time 3 mean.

Experiment 3:

For experiment 3, multivariate analysis of variance of the data indicates that there was a significant [time (T) x temperature (TE) x anticoagulant (AC)] interaction ($P=.025$) (Table 10). No overall statement could be made about any of the three main effects T, TE, and AC since each is involved in a significant interaction. When a significant interaction occurs, the experiment has to be broken down into separate parts for analysis. These analyses were done at each time period (Table 11). Each analysis was for a two-factor (anticoagulant and temperature) experiment with repeated measures on both factors. At time 0, there was a significant difference in the effect of anticoagulant ($p<.001$) with heparin samples exhibiting the higher mean value. There were no differences in COHb levels as a result of storage temperature. At time 3, there was a significant

difference in both anticoagulant ($p<.001$) and temperature ($p<.001$) effect. The mean for samples with heparin was higher than the means of those samples using EDTA and samples stored at 21°C had a higher mean than the mean of samples kept at 4°C. At time 5, the AC x TE interaction was significant ($p=.009$). Bonferroni t-tests (a multiple comparison procedure) were done on the six possible pairs of the four means to determine the cause of the AC x TE interaction. It could be concluded from these tests that the mean for EDTA at 21°C was significantly lower than the other three means and that these three means were not significantly different from each other ($p<.05$) (Figure 1).

DISCUSSION

Blood samples were stored under varying conditions to examine the stability of COHb in vacutainers, as measured spectrophotometrically by the IL-282 CO-Oximeter. The variables of interest were temperature, 4°C and 21°C; time, 0 to 14 days; anticoagulant, heparin and EDTA, and exposure to fluorescent room light. Three separate experiments were performed to determine these effects.

In the first experiment, where the subject's blood was used in only one of the four possible experimental anticoagulant/temperature combinations, measured over time, the results indicated that there was no effect of storage temperature or anticoagulant on the stability of COHb in stored blood samples for 14 days. There was; however, a statistically significant drop in COHb levels from day 0 to day 3. COHb values tended to then remain stable out to 14 days. The second experiment, where the subject's blood was divided across each cell by anticoagulant, wrapped/unwrapped, and time, while keeping temperature fixed at 4°C, the data indicated that there was no appreciable dissociation of CO from hemoglobin in the stored sample by ambient fluore-

scent light. Although light has been shown to cause the dissociation of carboxyhemoglobin, ambient room light in our laboratory was not intense enough to initiate this reaction (12). There was; however, a statistically significant decrease in COHb levels from day 0 to day 3 as seen in the first experiment and samples stabilized with heparin had statistically higher initial levels of COHb than samples containing EDTA as the anticoagulant.

The anticoagulant effect noted in the second experiment is not in agreement with the earlier findings on this effect in the first experiment. This can be explained by the difference in experimental designs used. In the first experiment, different subjects were used for each temperature and anticoagulant combination. Variability amongst subjects was a part of the error used to make the determination about the significance of differences between heparin and EDTA, as well as between storage temperatures. Because of the large differences of the initial COHb values between each subject, the experimental design used in the first experiment would most likely declare only large differences as significant.

The study design used in experiment two and three does not suffer from this defect since all combinations of effects were used on every subject, thereby allowing the removal of subject differences before testing for significance of effects. The experimental design used in the first experiment is therefore much less powerful for testing for an anticoagulant effect than in experiments two and three. This same weakness of experimental design is present in experiment one for the determination of the effect of storage temperature on COHb stability. It should be noted; however, that the design of the first experiment is better suited for determining changes in COHb due to length of storage time. This results from the fact that subject variability is of no consequence at each time interval. In

addition, the number of subjects is larger in experiment 1 (N=40) than in experiment 2 (n=9) and experiment 3 (N=11).

The third experiment addresses the shortcomings of experimental design used in experiment one to determine the effects of storage temperature on COHb stability. After three days of storage, all samples exhibited lower COHb values compared to samples obtained on day one. Samples maintained at 4°C, independent of anticoagulant used, had statistically significant lower COHb values than samples stored at 21°C; but only at day three. Data from experiments two and three indicate that samples which contained the anticoagulant heparin had 5 to 7% higher initial COHb levels than samples treated with EDTA.

At the end of five days of storage, a significant change was noted in COHb levels in experiment three, resulting from the choice of anticoagulant and storage temperature. At the end of three days storage, the greatest decrease in COHb was observed as previously noted in the first two experiments. The heparin treated samples decreased in value by 5 to 10%, of the original value while the EDTA treated samples declined by a factor of 6 to 11% of original value. In addition, there was an interaction between the choice of anticoagulant and temperature. An examination of the data revealed that the blood samples containing EDTA and stored at 21°C had significantly lower COHb values than the other three pairs of data. It was noted that in these samples, the methemoglobin (MetHb) levels were elevated well above those of the other samples. The mean value of MetHb increased from approximately 1% to a mean of 7.36 ± 4.99% (range of 0.9 to 14.77%). A decline in COHb levels in stored samples associated with an increase in MetHb has also been observed by Dahms (10). By comparison, Dennis and Valeri (11) demonstrated that COHb levels remain stable over a range of 0 to 70% MetHb. This discrep-

ancy may be explained by the fact that Dennis and Valeri generated MetHb artifically, using $K_3Fe(CN)_6$. Antonini and Brunori (12) point out that ferric hemoglobin prepared with ferricyanide may have small spectral differences when compared to ferric hemoglobin resulting from autoxidation as we have experienced. These small spectral shifts may result in larger changes in calculated COHb.

The results of these three experiments indicate that COHb levels measured by the IL-282 CO-Oximeter in blood samples stored in vacutainers containing either EDTA or heparin at (21°C) or (4°C) decay 5 to 11% within three days. Heparin treated samples decreased in value by 5 to 10%, while EDTA treated samples declined by 6 to 11% COHb. Additionally, it was noted that samples treated with EDTA as the anticoagulant started out with a 5 to 7% lower level of COHb than the heparin treated samples. Within each anticoagulant pair, the levels of COHb decayed at the same rate, regardless of the storage temperture. These results differ from studies reported by Collison et al. (6) where they have shown that blood samples treated with the anticoagulant heparin, oxalate and citrate were stable for ten days when stored at 21°C. Dahms and Horvath's work (5) has extended the length of sample stability out to three weeks at room temperature (20-25°c) with heparin treated samples kept in the dark. Stewart et al. (4), using EDTA as the anticoagulant, demonstrated that samples stored at 4°C were stable for six weeks, whereas samples kept at 22°C were stable for a two week period.

In the studies of Collison et al. (6) and Dahms and Horvath (5), the gas chromatographic method for the detection of CO was used. This procedure is independent of any spectral changes that may occur during storage and is

dependent upon the amount of CO that is dissociated from the hemoglobin molecule. The data of Stewart et al. (4) was based upon the same technique that was employed in this study, but used an IL-182 model CO-Oximeter, an older model, to estimate COHb. Although Stewart et al. (4) do not present data to support the stability of blood samples, they provided an estimate of their measurement variability, using a single sample with repeated measures over a sixteen day period. A mean COHb value of 1.2% was reported with a standard error of 0.13% and a range of 1.0% to 1.4%. Our precision is routinely 0.1% lower on repeated measures.

This entire issue of COHb stability in stored samples is a very complex one. Whereas autoxidation is facilitated by an increase in temperature, addition of EDTA, acting as a chelating agent, slows the autoxidation process (12). These effects along with possible variations in sample pH tend to alter the spectral characteristics of the various hemoglobin complexes, often in opposing directions.

CONCLUSIONS

1. There is a need for additional studies to more thoroughly define the degradation of COHb during storage over three days. The optimum time for COHb analyses using the IL-282 co-oximeter has not been determined.
2. Ambient Fluorescent room light and room temperature had no effect on the determination of COHb under conditions of this study. No additional testing is required to confirm this conclusion.
3. Further studies are required to determine why there is a difference in COHb measurements when the anticoagulant heparin and EDTA are used.

APPENDIX:

During the course of this study, we participated in an interlaboratory comparison study with the Defense and Civil Institute of Environmental Medicine (DCIEM) on two occasions. Our laboratory used the IL-282 Co-Oximeter to quantitate the levels of COHb in venous blood samples, while

the DCIEM laboratory used a gas chromatographic procedure to quantitate the levels of COHb.

In the first comparison, about two dozen samples were first analyzed in our laboratory, then shipped to the DCIEM laboratory. This transfer took over ten days to accomplish, possibly accounting for the lower values in the DCIEM determinations.

For the second comparison, we proposed the following study to address the possible decrease of COHb with time. During an upcoming DCIEM study, they were to take triplicate samples from each exposed subject. The DCIEM laboratory would measure one of three samples immediately, they would retain one sample, and ship the third sample to the EPA laboratory. Upon receipt of the samples at EPA, we would telephone the DCIEM laboratory and coordinate the time and date of the next analyses. In this manner, both laboratories would measure the paired samples at the same length of time after they were obtained. A comparison of the values taken initially to those obtained subsequently at DCIEM would give an estimate of sample stability (using the GC method).

The results obtained on sample comparisons from the second study showed the delta's of eight samples for COHb values ranging from <1% to >11% were 0 to ±0.8% COHb. These results show better agreement between the two laboratories than the initial comparison. Below 10% COHb, the differences were about equal and in the same direction indicating that the precision is good on both methods, leaving further research required to determine the accuacy of each procedure.

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TABLE 1

Experimental Design for the First Experiment. A three-factor experiment (temperature, anticoagulant, and time) with repeated measurements on time (N = 40 subjects)

TEMP	ANTI-COAGULANT	SUBJECT NUMBER	TIME (DAYS) 0	3	5	7	10	12	14
21°C	Heparin	1 ↓ ↓ 5 ↓ ↓ ↓ 10	COHb1[a] COHb2 COHb3						
	EDTA	11 ↓ ↓ 15 ↓ ↓ ↓ 20				COHb1 COHb2 COHb3			
4°C	HEPARIN	21 ↓ ↓ ↓ 25 ↓ ↓ 30							COHb1 COHb2 COHb3
	EDTA	31 ↓ ↓ 35 ↓ ↓ ↓ 40		COHb1 COHb2 COHb3					

[a] = triplicate analyses for each sample.

TABLE 2

Experimental Design for the Second Experiment. A three-factor experiment (anticoagulant, wrapped/unwrapped and time) with repeated measures on all factors. Samples were stored at 4°C.

ANTI-COAGULANT	HEPARIN						EDTA					
Wrapped or Unwarapped	Wrapped			Unwrapped			Wrapped			Unwrapped		
Time(days)	0	3	5	0	3	5	0	3	5	0	3	5
Subject 1	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6	Tube 7	Tube 8	Tube 9	Tube 10	Tube 11	Tube 12
SUBJECT 2												
SUBJECT 3												
SUBJECT 4												
SUBJECT 5												
SUBJECT 6												
SUBJECT 7												
SUBJECT 8												
SUBJECT 9												

TABLE 3

Experimental Design for the Third Experiment. A three-factor experiment (temperature, time, and anticoagulant) with repeated measures on all factors.

ANTI-COAGULANT	HEPARIN						EDTA					
Temperature	4°			21°			4°			21°		
Time(days)	0	3	5	0	3	5	0	3	5	0	3	5
Subject 1	Tube 1	Tube 1	Tube 1	Tube 2	Tube 2	Tube 2	Tube 3	Tube 3	Tube 3	Tube 4	Tube 4	Tube 4
SUBJECT 2												
SUBJECT 3												
SUBJECT 4												
SUBJECT 5												
SUBJECT 6												
SUBJECT 7												
SUBJECT 8												
SUBJECT 9												
SUBJECT 10												
SUBJECT 11												

TABLE 4

Multivariate Analysis of Variance of the Factors, Anticoagulant, Temperature, and Time

SOURCE	F-Test Degrees of Freedom	P value
ANTICOAGULANT (A)	1, 36	.244
TEMPERATURE (T)	1, 36	.768
A x T	1, 36	.130
TIME	6, 31	<.001
A x TIME	6, 31	.258
T x TIME	6, 31	.346
A x T x TIME	6, 31	.733

TABLE 5

Mean % COHb Values Across The Combinations of Temperature and Anticoagulant Over Time

(N=10 for each mean)

TIME (DAYS)	TEMPERATURE AND ANTICOAGULANT COMBINATION							
	HEPARIN 4°C		HEPARIN 21°C		EDTA 4°C		EDTA 21°C	
	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.
0	4.81	0.77	6.13	1.49	5.21	1.28	3.18	0.49
3	4.29	0.78	5.77	1.51	4.82	1.27	2.92	0.50
5	4.30	0.79	5.71	1.54	4.68	1.29	2.88	0.53
7	4.29	0.82	5.66	1.52	4.61	1.27	2.86	0.47
10	4.53	0.78	5.68	1.52	4.65	1.30	2.54	0.37
12	4.41	0.74	5.67	1.49	4.70	1.28	2.50	0.41
14	4.12	0.75	5.54	1.50	4.75	1.31	2.69	0.37

TABLE 6

Mean % COHb of Pooled Values Across 14 Days of Storage.[a] N = 40.

	TIME(DAYS)						
	0	3	5	7	10	12	14
MEAN	4.83	4.45	4.39	4.36	4.35	4.32	4.27
S.E.	0.55	0.55	0.56	0.55	0.55	0.55	0.54

[a] In this experiment, there was no significant effect due to anticoagulant nor temperature. The only significant variable was time, therefore, pooling of the samples was justified.

TABLE 7

Estimates of the Variability of Measured COHb Over a Range of <2 to 17%

MEAN COHb	POOLED VARIANCE	POOLED STANDARD DEVIATION
<2	.02298	.152
2 to <3	.02600	.161
3 to <4	.01964	.140
4 to <5	.01767	.133
5 to <9	.03286	.181
$\geq$ 9	.03420	.185

H1: All 6 variances equal $X^2(5) = 14.35$, $p = .014$.

H2: First 4 variances equal $X^2(3) = 4.31$, $p = .230$.

H3: Last 2 variances equal $X^2(1) = 0.03$, $p = .872$.

COHb < 5: $s^2 = .02181$ (420 d.f.), $s = .148$.

COHb $\geq$ 5: $s^2 = .03338$ (138 d.f.), $s = .183$

TABLE 8

Multivariate Analysis of Variance of the Factors Anticoagulant, Time and the Effect of Ambient Light Levels on the Dissociation of COHb.

SOURCE	F-Test D.F.[a]	P Value
TIME (T)	2,7	.042
WRAPPED OR UNWRAPPED (WU)	1,8	.914
ANTI COAGULANT (AC)	1,8	<.001
T X WU	2,7	.704
T X AC	2,7	.239
WU X AC	1,8	.702
T X WU X AC	2,7	.260

[a]Degrees of Freedom

TABLE 9

Mean COHb Values of Tubes Exposed/Blocked from Ambient Light. Comparison of Anticoagulant and Light Induced COHb Dissociation.

Anti-Coagulant	Wrapped or Unwrapped	Time	N	Mean	S.E.[1]	TRIPLICATE SAMPLE VARIANCE
Heparin	Wrapped	0	9	4.36	.083	.1107
		3	9	4.09	.083	.0507
		5	9	4.12	.083	.0456
	Unwrapped	0	9	4.34	.083	.0544
		3	9	4.05	.083	.0807
		5	9	4.20	.083	.0470
EDTA	Wrapped	0	9	4.04	.083	.0437
		3	9	3.77	.083	.0930
		5	9	3.93	.083	.0522
	Unwrapped	0	9	4.09	.083	.0444
		3	9	3.73	.083	.0363
		5	9	3.86	.083	.0218

Means of Significant Main Effects

Variable	N	Mean	S.E.[1]
Time:			
0	36	4.21	.042
3	36	3.91	.042
5	36	4.02	.042
Anti-Coagulant			
Heparin	54	4.19	.034
EDTA	54	3.90	.034

[1] For determining differences in means

TABLE 10

Multivariate Analysis of Variance of the Factors Time, Temperature, and Anticoagulant on COHb Stability.

ANALYSIS OF VARIANCE

SOURCE	F-test degrees of freedom	P Value
TIME (T)	2,9	<.001
TEMPERATURE (TE)	1,10	.789
ANTI COAGULANT (AC)	1,10	<.001
T X TE	2,9	<.003
T X AC	2,9	.224
TE X AC	1,10	.273
T X TE X AC	2,9	.025

TABLE 11

Separate Analysis, by Time, of the Interaction of Anticoagulant and Temperature on COHb Stability

	P VALUE		
SOURCE	TIME 0	TIME 3	TIME 5
ANTICOAGULANT (AC)	<.001	<.001	<.001
TEMPERATURE (TE)	0.999	<.001	0.045
AC X TE	0.811	0.201	0.009

TABLE 12

INTERLABORATORY COMPARISON

FIRST COMPARISON

Sample #

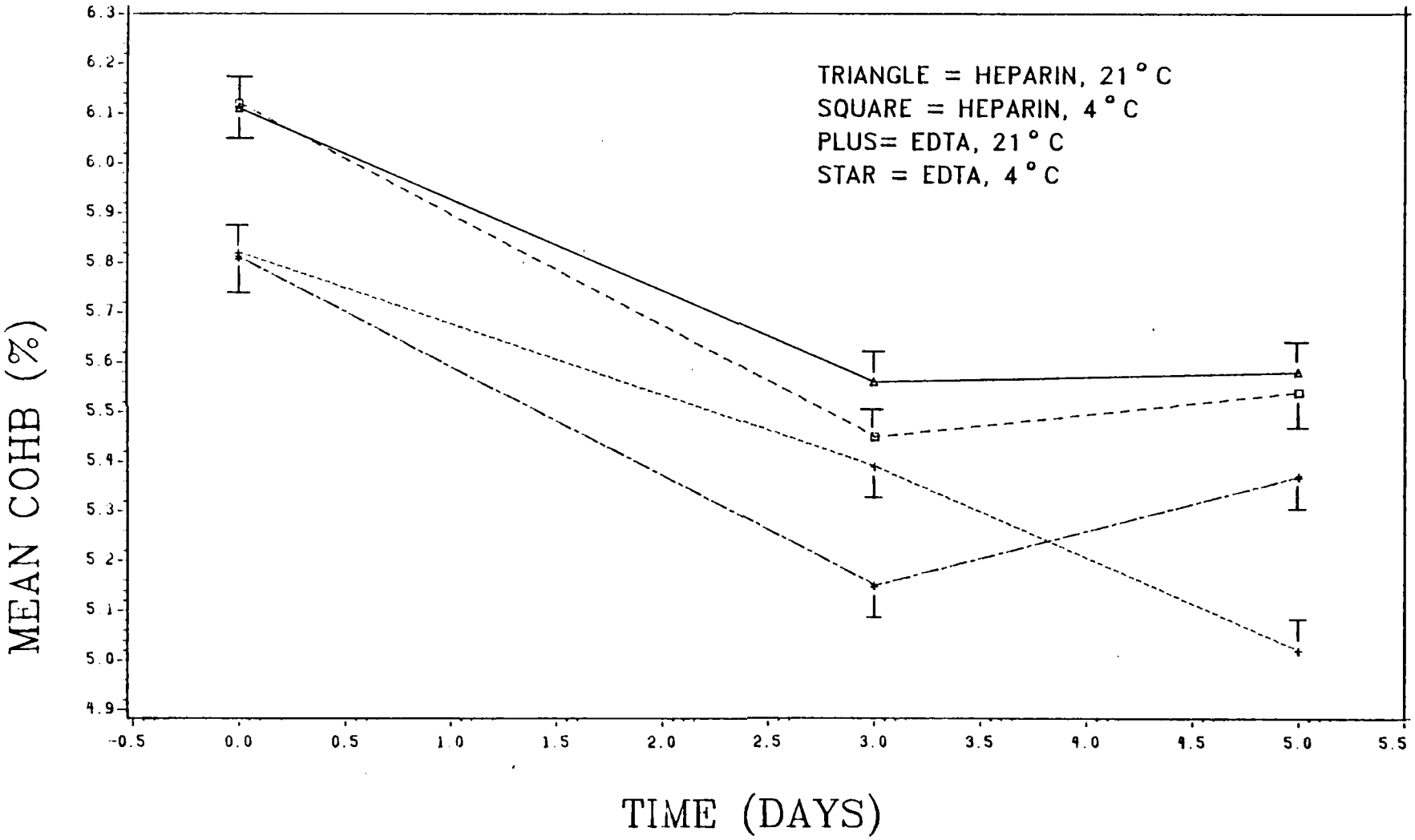
	5-11-07PO	5-27-06PR	5-27-06PR	5-11-07PR	5-39-83PR
DCIEM (gas chromatograph)	4.8	1.5	4.9	<1	1.0
USEPA (Co-Oximeter)	5.7	2.2	6.0	0.7	0.2
Difference (Δ) between labs	+0.9	+0.7	+1.1		-0.8

SECOND COMPARISON

Sample #

Laboratory	MD-1	MD-2	MD-3	MD-4	L-1	L-2	L-3	L-4
DCIEM (gas chromatograph)	<1	4.7	3.7	8.2	<1	6.7	6.0	11.9
USEPA (Co-Oximeter)	0.6	4.2	2.9	7.8	0.4	6.3	6.0	12.3
Difference(Δ) between Labs		-0.5	-0.8	-0.4		-0.4	0	+0.4

FIGURE 1. EFFECT OF ANTICOAGULANT AND TEMPERATURE ON COHB LEVELS (MEAN ± SEM) IN STORED BLOOD SAMPLES



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